REMARKS

I. Status of the Claims

Claims 251-287 and 625 were pending in the June 21, 2011 Office Action. With this reply, claim 625 is canceled. The cancelation of claim 625 is made without prejudice or disclaimer. Claims 251-287 are presented for reconsideration.

II. Information Disclosure Statement

Provided herewith is an Information Disclosure Statement (IDS) under 37 CFR 1.97(c), citing six references. The IDS fee under 37 CFR 1.17(p) is also provided herewith. Applicants ask the PTO to consider the references provided in the enclosed IDS when evaluating the patentability of the pending claims.

III. Rejections under 35 U.S.C. § 103

(a) Claims 251, 252, 254,256, 259-264, 269-273, 275, 281, 284-287 and 625 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lin et al. (US 6,197,554) in view of Nam et al. (2002, Proc. Natl. Acad. Sci. USA 99:6152-6156) and in further view of Laird et al. (EP 1 201 768). Applicants request reconsideration and withdrawal of this rejection in light of the following comments.

Lin et al. is again asserted to teach all elements of the claims except "…that the primers contain 3' terminal nucleotides that are substituted with nucleotide analogues having a modification at the 2' position of the ribose ring …." Office Action at p. 7. Laird et al. is asserted to teach "methods for conducting PCR amplification using modified primers" including 2'-O-methyl-nucleotides, 2'-fluoro-nucleotides, and 2'-amino-nucleotides (Office Action at pp. 8) and Nam et al. is asserted to provide motivation to combine Lin et al. and Laird et al. to apply the modified primers of Laird et al. to the primers of Lin et al. (Office Action at p. 9).

The Office Action also asserts at p. 8 that Nam et al. provides the requisite motivation by teaching that "…oligo(dT) primers can produce spurious truncated amplification products during reverse transcription reactions due to their ability to

hybridize to internal polyA sequences contained in the mRNA template in addition to the polyA tail of the mRNA template…."

In response to Applicants' argument that the use of nucleotide analogs at the 3' end of the primers in the claimed invention is to prevent unextended primers from undergoing addition of a non-inherent UDT, which neither Laird et al. nor Lam et al. address, the Office Action asserts at p. 24-25 that the skilled artisan would nonetheless

> …have been motivated to modify the oligo(dT) primers of Lin to include the additional one or two target-specific 3'-terminal nucleotides taught by Nam to be useful in reducing the production of truncated amplification products stemming from undesired priming from internal polyA sequences. Then, since Nam taught that the disclosed anchored oligo(dT) primers reduced, but did not eliminate, the production of undesirable truncated amplification products during reverse transcription reactions…, the ordinary artisan would have been motivated to additionally modify the primer such that one to three of the 3'-terminal nucleotides were 2'-O-methyl-nucleotides, 2'-fluoro-nucleotides, or 2'-amino-nucleotides in order to further improve the specificity of the amplification reaction as taught by Laird.

In response, Applicants reiterate that the skilled artisan would understand that there is no motivation for improving the specificity of the amplification reaction as taught by Laird, since there is essentially universal amplification of any and all sequences having a polyA tail anyway. Thus, even if Nam is adapted for use with the method of Lin et al., there is no particular motivation to add the method of Laird et al. since the problems identified in Laird et al., primer-dimer formation and spurious expression results derived from priming at inappropriate sequences, are not problems in the method of Lin et al. It is only in the present application that particular benefits are shown to be endowed to a universal RNA amplification system for expression analysis by the presence of nucleotide analogues at the 3' end of primers. Thus, there is no reason to combine Lin et al. and Laird et al. because the problem identified in Laird et al. is not present in the Lin et al. method.

The present invention is not drawn to the use of modified primers to "reduce non-specific amplification of the target nucleic acid". Rather, the use of modified primers in the present invention solves two problems associated with the steps of that are particular to the claimed methods: 1) terminal transferase addition of nucleotides to

primers that have **not been extended** by use of target mRNA templates and 2) the use of **unextended** primers for promoter independent transcription by T7 RNA polymerase. Neither of these problems is associated with PCR *per se* but rather they are germane to the claimed methods. Both of these types of nucleic acid synthesis results in target independent nucleic acid synthesis that would generate labeled material irrelevant to any analytes in the reaction. Consequently, Applicants have discovered that the use of modified nucleotides at the 3' terminus of primers can reduce these aberrant forms of nucleic acid synthesis. It can be readily seen that these effects are not related to inappropriate extension reactions, which is the heart of the advantages described by Laird et al. for application of modified primers in PCR. Consequently, the statement on page 9 of the Office Action that "it is *prima facie* obvious to apply known methods to a similar method to improve the method in the same way" is inappropriate since the application of modified primers in the present invention does not "improve the method in the same way" described by Laird.

The skilled artisan would also understand that the modified primers described by Laird et al. would not be useful with the methods of Lin et al. as described therein. The Office Action, at p. 8, discusses the mechanism offered by Laird et al. for the success of their method with PCR as "Laird teaches that the disclosed modified primers increase the time required for the initial primer extension [e.g., with improper internal polyA sequences], and thereby reduce non-specific amplification of the target nucleic acid...." This is a reasonable explanation of how benefits could be conveyed, since the stepwise manner of performing PCR leaves a limited window for an extension to take place before there is a subsequent denaturing event. In the examples given by Laird et al. were the modified primers were useful, the annealing/extension step at 65 °C lasts for 40 seconds before a denaturation step of 95 °C in Examples 1, 4 and 5, and for 60 seconds in Example 7. With inappropriate bindings slowed down by the use of modified primers, the skilled artisan would understand that a PCR cycle would be ended before an inappropriate extension could take place. Thus, in using the modified primers in a succession of cycles, each cycle with an internal polyA sequence would likely end before non-target extensions can accumulate. However, this kinetic rationale for the

use of modified primers in Laird et al. does not apply to the essentially isothermal reaction described in Lin et al., where the annealing/extension of a primer to a homopolymeric sequence takes place over a period of 3 hours (see Examples 2 and 4 of Lin et al.) In the qualitatively different time scale taught by Lin et al., the increased time it takes for the modified primers to anneal and be extended does not affect any inappropriate binding that might take place, because the long incubation time would allow annealing and extension of the modified primers on improper internal polyA sequences anyway. Thus, the purpose for using the modified primers as taught by Laird et al. is not applicable to the long incubation times taught by Lin et al. since the kinetic effect presented by Laird et al. would not generate any significant improvements during the 3 hour incubation taught in Lin et al. This provides another reason why there is no motivation to combine the methods of Laird et al. with Lin et al., since the effects of the modified primers as described by Laird et al. would not be effective for the Lin et al. methods. Indeed, the mechanism described by Laird et al. as to how the modified primers are effective in preventing inappropriate priming and extension teaches away from any utility for the modified primers in reactions that have the long annealing/extension times described in Lin et al. Benefit for modified primers in methods that lack the short annealing/extension steps of PCR is thus first described in the instant specification, where a surprising and unexpected reduction in target-independent synthesis is achieved with the use of primers having modifications at the 3' end.

Since there is no motivation to combine the cited references, the instant obviousness rejection has no basis. Withdrawal of this rejection is therefore respectfully requested.

(b) Claims 253, 255, 257 and 258 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lin et al. (discussed under **(a)** above) in view of Nam et al. (discussed under **(a)** above) and further in view of Laird et al. (discussed under **(a)** above) and in further view of Kutsu et al. (US 6,242,189). The Office Action asserts at p. 12 that the combination of Lim et al., Nam et al. and Laird et al. teach or suggest all

elements of claims 253, 255, 257 and 258 except "...that the library of nucleic acid targets is comprised of copies of nucleic acids isolated from a biological sample ..." and "...adding a homopolymeric sequence to the library of nucleic acid targets using an enzyme, such as TdT, after isolation of the nucleic acids from the biological sample...." Applicants request reconsideration and withdrawal of this rejection in light of the following comments.

As discussed under **(a)** above, the methods of Lin et al., Nam et al. and Laird et al. cannot be combined since the use of the modified primers as taught by Laird et al. would not work with the methods of Lin et al. Kutsu et al. also do not provide a motivation to combine those references, since Kutsu et al. do not discuss modified terminal nucleotides for solving problems in methods of copying libraries using primers to homopolymeric sequences. Thus, the combination of Lim et al., Nam et al., Laird et al. and Kutsu et al. do not teach or suggest the claimed method. As such, those references do not render the instant claims obvious. Withdrawal of this rejection is therefore respectfully requested.

(c) Claims 265-268 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lin et al. (discussed under **(a)** above) in view of Nam et al. (discussed under **(a)** above) and further in view of Laird et al. (discussed under **(a)** above) and in further view of Willis et al. (US 6,858,412) and further in view of Moran et al. (1996, Nucleic Acids Research 24:2044-2052). The Office Action asserts at p. 14 that the combination of Lim et al., Nam et al. and Laird et al. teach all elements of claims 265-268 except for "...a terminator nucleotide in the TdT tailing reaction...." Applicants request reconsideration and withdrawal of this rejection in light of the following comments.

As discussed under **(a)** above, the methods of Lin et al., Nam et al. and Laird et al. cannot be combined since the use of the modified primers as taught by Laird et al. would not work with the methods of Lin et al. Neither Willis et al. nor Moran et al., alone or in combination with any of the other cited references, provide the lacking motivation since those references do not discuss modified terminal nucleotides for solving

problems in methods of copying libraries using primers to homopolymeric sequences. Thus, the combination of Lim et al., Nam et al., Laird et al. and Willis et al. and Moran et al. do not teach or suggest the claimed method. As such, those references do not render the instant claims obvious. Withdrawal of this rejection is therefore respectfully requested.

(d) Claims 274 and 276 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lin et al. (discussed under **(a)** above) in view of Nam et al. (discussed under **(a)** above) and further in view of Laird et al. (discussed under **(a)** above) and in further view of Sousa et al. (US 5,849,546). The Office Action asserts at pp. 16-17 that the combination of Lim et al., Nam et al., and Laird et al. teach or suggest all elements of claims 274 and 276 except for "...the use of a mutated RNA polymerase for generation of a chimeric RNA/DNA transcript...." Applicants request reconsideration and withdrawal of this rejection in light of the following comments.

As discussed under **(a)** above, the methods of Lin et al., Nam et al. and Laird et al. cannot be combined since the use of the modified primers as taught by Laird et al. would not work with the methods of Lin et al. Sousa et al., alone or in combination with any of the other cited references, do not provide the lacking motivation since Sousa et al. do not discuss modified terminal nucleotides for solving problems in methods of copying libraries using primers to homopolymeric sequences. Thus, the combination of Lim et al., Nam et al., Laird et al. and Sousa et al. do not teach or suggest the claimed method. As such, those references do not render the instant claims obvious. Withdrawal of this rejection is therefore respectfully requested.

(e) Claims 277, 278 and 280 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lin et al. (discussed under **(a)** above) in view of Nam et al. (discussed under **(a)** above) and further in view of Laird et al. (discussed under **(a)** above) and in further view of Steffens et al. (1995, Genome Research 5:393-399). The Office Action asserts at p. 18 that the combination of Lim et al., Nam et al., and Laird et al. teach or suggest all elements of claims 277, 278 and 280 except for "...including

labeled nucleotides in the final RT-PCR amplification step used to generate a copy of the RNA transcription product..." or specific examples of labeled nucleotides. Applicants request reconsideration and withdrawal of this rejection in light of the following comments.

As discussed under **(a)** above, the methods of Lin et al., Nam et al. and Laird et al. cannot be combined since the use of the modified primers as taught by Laird et al. would not work with the methods of Lin et al. Steffens et al., alone or in combination with any of the other cited references, do not provide the lacking motivation since Steffens et al. do not discuss modified terminal nucleotides for solving problems in methods of copying libraries using primers to homopolymeric sequences. Thus, the combination of Lim et al., Nam et al., Laird et al. and Steffens et al. do not teach or suggest the claimed method. As such, those references do not render the instant claims obvious. Withdrawal of this rejection is therefore respectfully requested.

(f) Claim 279 is rejected under 35 U.S.C. 103(a) as being unpatentable over Lin et al. (discussed under **(a)** above) in view of Nam et al. (discussed under **(a)** above) and further in view of Laird et al. (discussed under **(a)** above) and in further view of Sousa et al. (discussed under **(d)** above) and in further view of Steffens et al. (discussed under **(e)** above). The Office Action asserts at p. 20 that the combination of Lim et al., Nam et al., Laird et al. and Sousa et al. teach or suggest all elements of claims 279 except for specific examples of labels, which is provided by Steffens et al. Applicants request reconsideration and withdrawal of this rejection in light of the following comments.

As discussed under **(a)** above, the methods of Lin et al., Nam et al. and Laird et al. cannot be combined since the use of the modified primers as taught by Laird et al. would not work with the methods of Lin et al. Sousa et al. and Steffens et al., alone or in combination with any of the other cited references, do not provide the lacking motivation since those references do not discuss modified terminal nucleotides for solving problems in methods of copying libraries using primers to homopolymeric sequences. Thus, the combination of Lim et al., Nam et al., Laird et al., Sousa et al.

and Steffens et al. do not teach or suggest the claimed method. As such, those references do not render the instant claims obvious. Withdrawal of this rejection is therefore respectfully requested.

(g) Claims 282 and 283 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lin et al. (discussed under **(a)** above) in view of Nam et al. (discussed under **(a)** above) and further in view of Laird et al. (discussed under **(a)** above) and in further view of Stinear et al. (1996, Applied and Environmental Microbiology 62:3385-3390). The Office Action asserts at p. 22 that the combination of Lim et al., Nam et al., and Laird et al. teach or suggest all elements of claims 282 and 283 except for "...the use of bead-immobilized primers..." which is taught by Stinear et al. Applicants request reconsideration and withdrawal of this rejection in light of the following comments.

As discussed under **(a)** above, the methods of Lin et al., Nam et al. and Laird et al. cannot be combined since the use of the modified primers as taught by Laird et al. would not work with the methods of Lin et al. Stinear et al., alone or in combination with any of the other cited references, do not provide the lacking motivation since that reference does not discuss modified terminal nucleotides for solving problems in methods of copying libraries using primers to homopolymeric sequences. Thus, the combination of Lim et al., Nam et al., Laird et al. and Stinear et al. do not teach or suggest the claimed method. As such, those references do not render the instant claims obvious. Withdrawal of this rejection is therefore respectfully requested.

(h) Claim 625 is rejected under 35 U.S.C. 103(a) as being unpatentable over Nam et al. (discussed under **(a)** above) in view of Laird et al. (discussed under **(a)** above). This rejection is moot since claim 625 is canceled.

IV. Conclusion

In view of the foregoing remarks, Applicants respectfully request withdrawal of the rejections of record and passage of the claims to allowance.

Applicants authorize the United States Patent and Trademark Office to charge all fees required to maintain pendency of this application, including the extension of time, Request for Continued Examination, and IDS fees, to Deposit Account No. 05-1135.

If a telephone conversation would further the prosecution of the present application, Applicants' undersigned attorney requests that he be contacted at the number provided below.

Respectfully submitted,

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